THE ORIGIN OF VARIATIONS IN SEXUAL AND SEX-LIMITED CHARACTERS

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IN dealing with sex and its determination, attention has been most sharply focused upon forms with separate sexes and upon the visible differences between the chromosome groups of the two sexes. The result has been that the formulation of sex-determination has remained in terms of chromosomes, while the modern unit of determination is the gene; and also the subject of sex has been rather separated off from the main body of heredity. My discussion will be largely a process of resolving chromosomes into component genes, and showing that the conception of the nature and action of genes as gained from the study of non-sexual characters is valid in interpreting sex phenomena.

The facts of mutation and of linkage have given us the conception of a gene as a distinct chemical entity having a definite location in a particular chromosome. Each gene is essentially a factory, which is manufacturing a characteristic set of chemical products that are delivered to the common cytoplasm, and that produce development through interaction with each other and with materials from outside. But since the chemicals produced by the different genes are different, some genes will have much effect upon one character and little effect upon another, so that a relatively small proportion of the genes will be actively concerned in producing any given character. Some of these genes tend to make the character more pronounced, and others tend to make it less pronounced, so that the grade of development actually realized by each particular character will be determined by the equilibrium between its modifying genes. The forms into which a given character can be modified are in general quite diverse, but for the sake of simplicity we may call them all plus or minus modifications. If the effectiveness of a given plus or minus modifier is changed by mutation, the grade of the character will shift correspondingly.

We can conceive of the evolution of the sexual

characters of hermaphrodites in terms of successive simple mutations in genes. But to interpret male and female forms with observed differences in number or size of chromosomes and with sex-linked inheritance requires comparison with mutations in which the unit of change is a whole chromosome or section of chromosome instead of a single gene. Such mutations can be understood in terms of the action of component genes as follows. Linkage experiments show that the various kinds of genes are distributed pretty much at random among the various chromosomes and along each chromosome. But since the number of genes with a given tendency is relatively small, any particular small section of chromosome might not contain these genes in the same proportion as they exist in the entire complement, and still less would the normal proportion of every kind be present. The loss of a section of chromosome (a condition known as deficiency) would ordinarily remove more minus than plus modifiers (or vice-versa), and since in that case more plus than minus modifiers would remain in action, the grade of the corresponding character would be shifted in a minus direction. This is the interpretation of the fact that a deficiency may cause many character changes, the complex of altered characters being inherited as a dominant. When a whole chromosome is lost through nondisjunction, the effects are similar to those in deficiency for a section except that they are greater in degree.

The way in which genes act together in producing a character, and the relation of the balance of plus and minus modifiers to deficiency or to the absence of a chromosome may perhaps be made clearer by an analogy. Let us suppose that a man is an ardent stamp collector, and has accumulated a lot of stamps. These stamps are to represent genes, so their number may be put at 5,000 to correspond roughly to the number of genes in *Drosophila*. Among the Russian stamps, especially those of recent issue, there is a very large number of reds, but also a fair number of pinks, and even a few whites. These differences in tint correspond to the plus and minus modifiers of a certain character, namely, the redness of Russian stamps. Now the stamps of different tints are in some definite ratio, whatever that ratio is, and we will call it the

normal ratio or balance. This stamp collector carries his collection around with him, and it fills two big, coat pockets, a trousers pocket, and there are even a few in his vest pocket. But unlike most collectors this one has never taken the trouble to sort over more than a few of his stamps. Meanwhile he strings them together pretty much hit or miss. This stringing stamps together is rather disapproved of by some other stamp collectors, who think that is no way to treat stamps, and each of whom has his own favorite method of arranging them. Because of this hit or miss method of making the strings of stamps the ratio among the different grades of redness of Russian stamps is different in different parts of the strings, and so if some other ardent collector should snip off a piece of one of the strings and carry it away, the remainder of the Russian stamps might have a considerably redder tone, while at the same time the Polish stamps might become bluer. If a whole string were lost, then many of the sets of stamps might have quite different complexions.

Now I have been recently studying the effects of the loss of one of the chromosomes of *Drosophila*,[1] namely, the small round fourth-chromosome, and the phenomena offer striking parallels to those of dicœcious sex, including sex-linked inheritance and sex-limited characters. Individuals having only one fourth-chromosome show a change in many characters, among which may be mentioned smaller size, smaller bristles, later hatching, poorer viability, paler body-color, darker trident pattern, shorter blunter wings, etc. Each of these differences corresponds to a character for which the fourth-chromosome was internally unbalanced, that is, for which the ratio of plus to minus modifiers was different from that of the whole group. For all of the characters in which there was an internal preponderance of plus modifiers the grade will be shifted in a minus direction by the loss of the fourth-chromosome, for example, the shorter wings and paler body-color. Likewise the characters that shift in a plus direction, as the darker trident pattern and the large eyes, are characters for which the fourth-chromosome possesses an excess of minus modifiers. In the

[1] *Proc. Nat'l Acad. of Sci.*, 7: 186–192.

male of *Drosophila* there is only one X-chromosome, though there is present a Y-chromosome that can be disregarded, since the evidence from non-disjunction of the X-chromosome shows that it has very little effect upon sex or characters. These individuals with only one X-

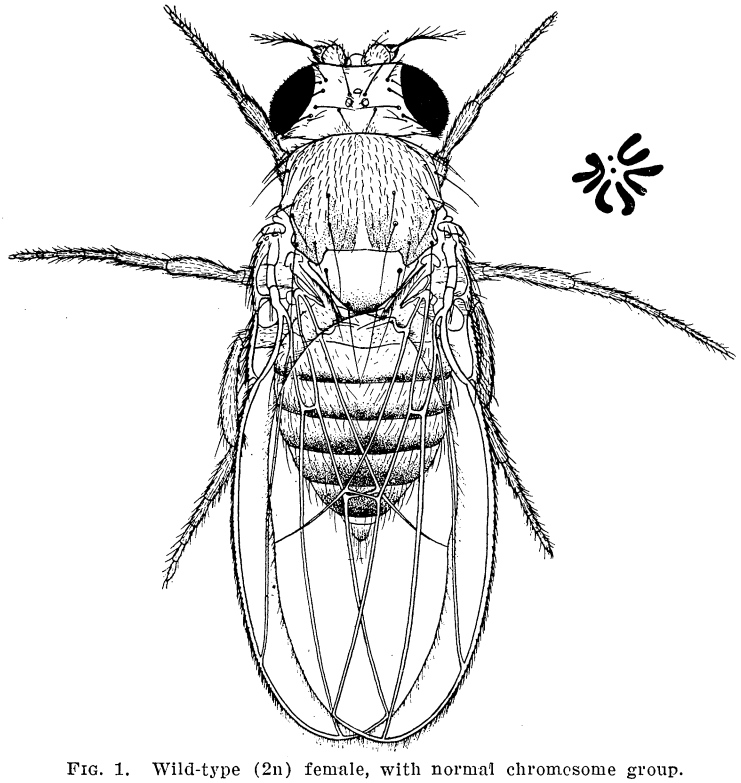

FIG. 1. Wild-type (2n) female, with normal chromosome group.

chromosome likewise show a complex of characters that are different from those shown by the individuals with the normal two X-chromosomes. Among these characters are gonads and genitalia of a type that we call male. The haplo-X individual is also smaller, has smaller bristles, is less viable, hatches later, and differs in other details from the 2-X type that we call female. Each of these differences likewise corresponds to a character for which the balance of the genes in the X is different from that in the group as a whole. The absence of one X leaves in action an unbalanced set of genes which produces male characters. The X-chromosome is a chromosome that is internally unbalanced by an excess of genes that we may call female-producing.

In an outcross of a haplo-IV individual to a normal, the entire complex of characters is inherited as a simple dominant and gives a 1 : 1 ratio, except that the haplo-IV's are less viable. Likewise in outcrosses of haplo-X individuals the entire complex of male characters is in-

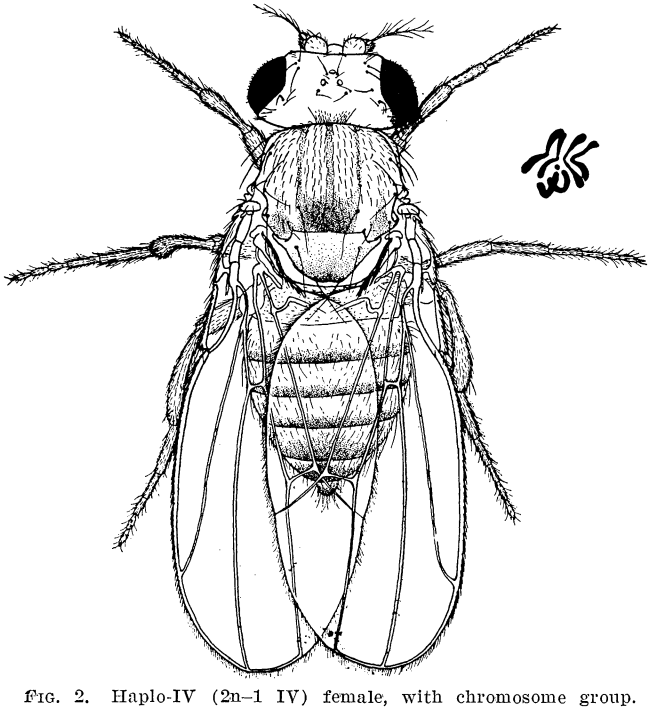

FIG. 2. Haplo-IV (2n–1 IV) female, with chromosome group.

herited as a simple dominant and gives a 1 : 1 ratio except that the haplo-X's are somewhat less viable.

When a haplo-IV individual is mated to a recessive whose gene is in the fourth-chromosome, all the haplo-fourth offspring show this recessive—a behavior that is strictly parallel to sex-linked inheritance; for if a haplo-X individual, that is, a male, is mated to a recessive whose gene is in the X, all the haplo-X offspring show this recessive.

The fourth-chromosome recessive characters present in haplo-IV individuals from the cross of a haplo-fourth to the recessive show a grade of development that is different from their grade as homozygous characters in diplo-IV's. This phenomenon is known as "exaggeration," and is interpreted as the effect of an unbalance within the

normal fourth-chromosome. With respect to a character that is exaggerated in a plus direction the fourth-chromosome has an unbalance in the minus direction. But since the whole complement is in balance, this unbalance within the fourth-chromosome is neutralized by a reciprocal unbalance in the other chromosomes. So the removal of one fourth-chromosome with its excess of minus modifiers leaves the remainder of the genes with an excess of plus modifiers, and these plus modifiers are free to work in the same direction as the recessive gene that is present, and thus to give an even greater effect than the homozygous recesive. Corresponding to these exaggerated fourth-chromosome characters there is a class of sex-linked characters that are exaggerated in the absence of one X-chromosome. These mutant characters show a different grade of development in the male from that which they show in the female. A good example is the race called eosin, in which the male has a much paler eye-color than the eosin female. These characters exaggerated by the absence of an X are called sex-limited. Some of them, like eosin, are exaggerated in a plus direction, corresponding to an excess of minus modifiers within the X-chromosome, while others, such as bobbed, are exaggerated in a minus direction. Thus bobbed, which shows scarcely at all in the males, corresponds to an excess of genes within the X tending to make bristles short, and two X-chromosomes can outweigh the genes in the autosomes that tend to make the bristles long, but one X is not enough to do so.

When haploidy for the fourth-chromosome is combined with mutants whose genes are outside the fourth-chromosome there is of course no effect corresponding to sex-linkage, but there is "exaggeration." Thus, haploidy for the fourth-chromosome exaggerates the third-chromosome mutant Hairless in a plus direction. This type of exaggeration finds its parallel in the 20 or so sex-limited mutations that are not sex-linked. These are mutations whose differential genes are in the autosomes and not in the X and which nevertheless show a different grade of development in the male from that in the female. In these cases also the modifiers of each character are of different weights in the X from the general collection,

and absence of one X leaves a surplus of genes that work in the same or in the opposite direction from that of the mutant in question.

Thus, by studying three kinds of effects, first, the character complexes that result directly, secondly, the exaggerations of the mutant characters whose genes are in the same section or chromosome as that involved in the loss, and thirdly the exaggerations of mutant characters whose genes are in other regions, we can analyse roughly the kinds and the signs of the genes that are in the region in question.

Since sexual and sex-limited characters are shown to rest on the same genetic basis, namely, a preponderance within the X of the plus or the minus modifiers of those characters, it may be questioned whether there is any real difference between these two categories. If the race of the mutant eosin were to become established in nature, a systematist would certainly include this difference in eye-color among his sexual differences. I am of the opinion that there is no difference between these two categories except that we call those sexual that are most closely connected with reproduction.

There is one striking difference between haploidy for X and haploidy for an autosome—namely, that the changes connected with haploidy for autosomes are relatively more numerous and extreme. Haploidy for the second or third autosomes probably produces changes so great as to be lethal, while haploidy for the very small fourth-chromosome produces changes comparable in extent to all those of the male aside from the reproductive organs. The proportion of sex-limited mutant characters is only about a tenth of the total, while X contains about a quarter of the genes. Since the changes in character produced by absence of an X are relatively small, the internal balance of the X must be relatively high. For a high proportion of the characters of the animal, the plus and minus modifiers in the X must be in about the same ratio as in the group as a whole.

The comparison just made between the effects of haploidy for an autosome and the effects normally present in diœcious sex shows that they have similar genic bases—namely, each is due to differences in the ratio between two

aggregates of genes; and that the X produces its characteristic effects because it contains a preponderance of genes tending to produce the characters that we call female. This point of view receives even stronger and more direct support from a study of cases in which the

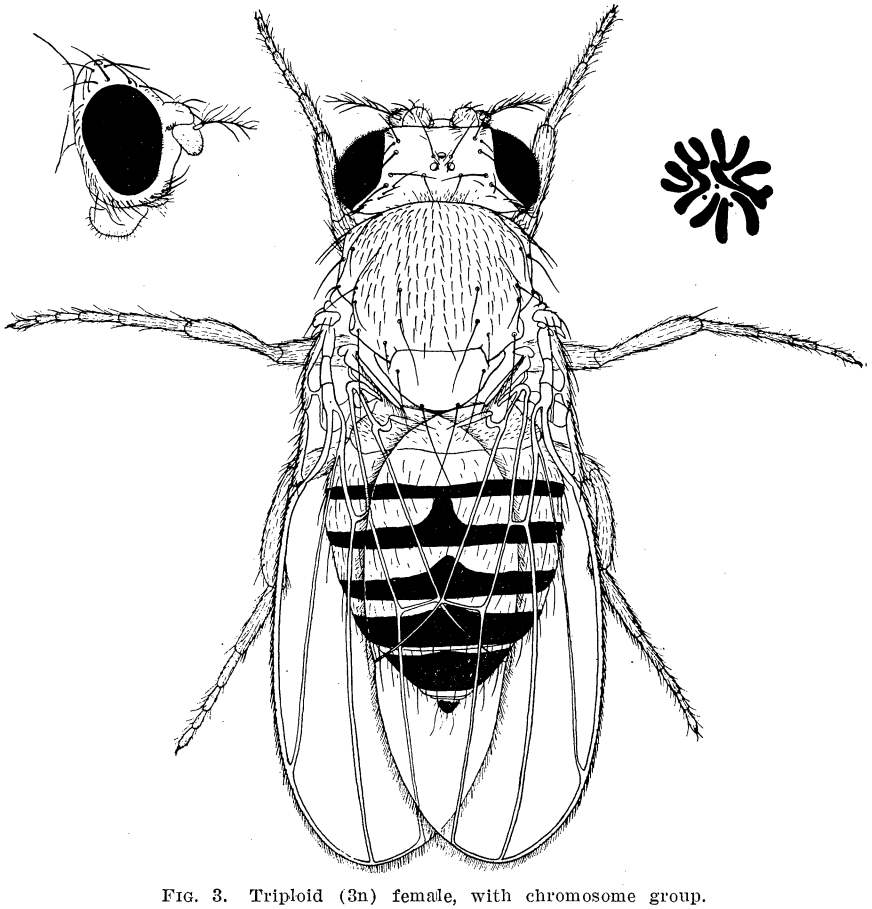

FIG. 3. Triploid (3n) female, with chromosome group.

ratio of X-chromosome to autosomes has been changed, and in which new sex relations are present. These new types of chromosome combinations and of sex take their origin in the occurrence of triploidy in *Drosophila,* for which there is full genetical and cytological proof.[2] The first point is that individuals having three full sets of chromosomes (3n) are females not to be distinguished from normal females except for slight differences in size and proportion that may well be due simply to the greater

[2] *Science,* N. S., 54: 252–254.

amount of chromatin. The nearly complete identity between the triploid and diploid forms both as to sex and as to non-sexual characters is a splendid evidence that these characters owe their grade to the ratios among the genes, for those ratios are identical in the 3n and 2n forms.

Among the offspring of triploid females are individuals that are neither males nor females but are sex-intermediates, or rather, are mixtures of male and female characters, very similar in type to the intersexes of *Lymantria.*[3] Genetical and cytological proof was obtained that these intersexes in *Drosophila* possess two X-chromosomes and three sets of autosomes. The old formulation of 2X equals ♀ is at once seen to be inadequate, for here we have individuals that have two X-chromosomes and yet are not females. They are shifted out of the female class by the presence of an extra set of autosomes, and thereby the autosomes are proved to play a positive rôle in the production of sex. Since the intersexes differ from females by the assumption of certain male characters this effect of the autosomes is due to an internal preponderance of "male-tendency" genes.

We may now formulate the sex-relations as follows: both sexes are due to the simultaneous action of two opposed sets of genes, one set tending to produce the characters called female and the other to produce the characters called male. These two sets of genes are not equally effective, for in the complement as a whole the female-tendency genes outweigh the male-tendency genes and the diploid (or triploid) form is a female. When the relative number of the female-tendency genes is lowered by the absence of one X, the male-tendency genes outweigh the female and the result is the normal haplo-X male. When the two sets of genes are acting in a ratio between these two extremes, as is the case in the ratio of *2X: 3 sets autosomes,* the result is a sex intermediate—the intersex.

The intersexes as a class can always be easily distinguished from normal males and females by reason of their large size, large coarse-textured eyes and by certain other characters such as scalloped wing-margins. Some of these characters are probably non-sexual effects of the

[3] R. Goldschmidt, *Zeit. f. ind. Abst. u. Vererb.,* 23: 1–199.

triploidy for the autosomes, others are sex-limited. Within the class of intersexes there is a very wide range of fluctuation, on the one hand to flies that are nearly female and on the other to flies that are entirely male in appearance. In an intersex of a given grade the several

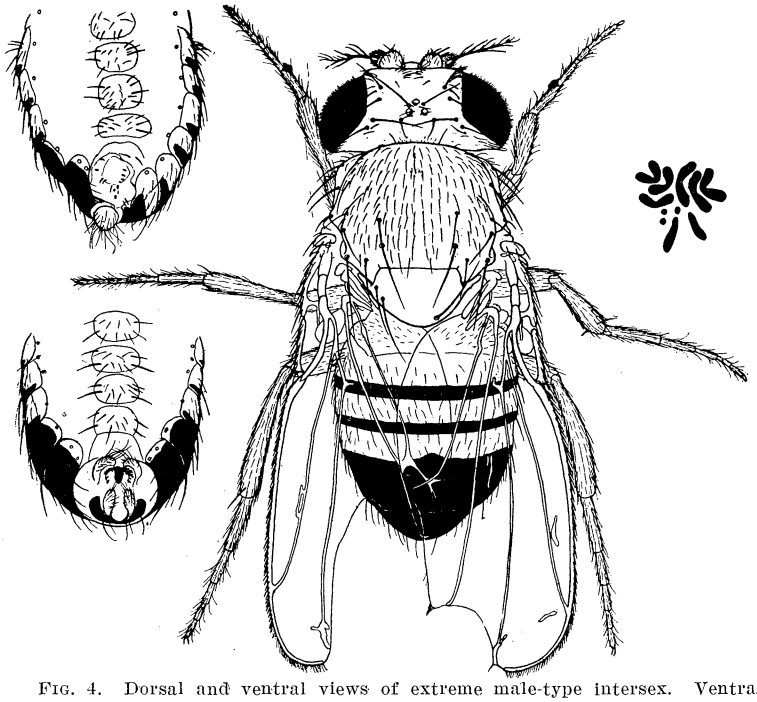

FIG. 4. Dorsal and ventral views of extreme male-type intersex. Ventral view of mid-grade intersex. Chromosome group of intersex showing 2X and 3 sets autosomes.

characters do not all present the same intermediate step between male and female, but, apparently just as in the intersexes of *Lymantria,* some characters are completely male, some completely female, while others are complex mixtures of male and female parts. When the intersexes are classified according to a system of grades, they are seen to be a bimodal class consisting of more "female-type" and more "male-type" intersexes, both of which fluctuate widely and overlap considerably.

The cytological investigation of the intersexes had shown that there are four sub-types of intersexes that differ in the presence or absence of a Y and in having three or only two fourth-chromosomes. It is possible, and there is some slight cytological and genetical evidence in support, that the male- and female-types of

intersexes correspond to the presence of three or of two fourth-chromosomes respectively.

There is another connection in which the wide fluctuations of the intersexes are interesting, namely, the action

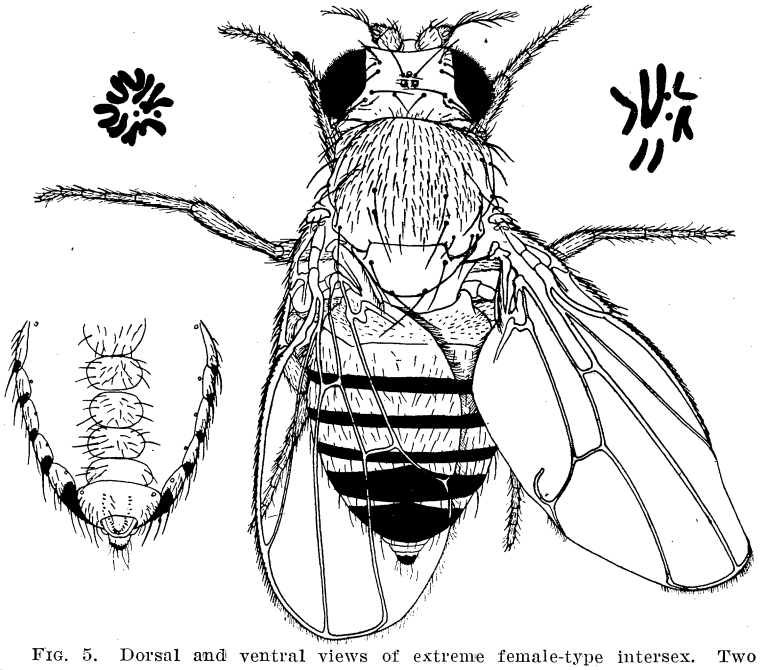

FIG. 5. Dorsal and ventral views of extreme female-type intersex. Two chromosome groups, the left with two IV-chromosomes and a Y, the right with two IV's but no Y.

of environmental factors. The slight range of fluctuation in such a character as miniature-wings in *Drosophila* probably means that there is a critical balance or ratio of plus to minus modifiers beyond which all balances give miniature, at least until the overbalance proceeds so far that a new critical ratio is passed and a new super-miniature character is realized. The balance in miniature is so far beyond the critical balance that only rarely are the environmental factors strong enough to outweigh this overbalance and thus cause fluctuation. In mutants in which the overbalance is slight there will be both wide fluctuation due to environmental interference and a high susceptibility to modification by other genes, as is notoriously the case with Beaded and with Truncate.

In normal males and females there are high overbalances beyond the critical points, and consequently only slight genetical or fluctuating variations. But in the in-

tersexes these two overbalances in opposite directions cancel each other, and since the two sets of genes are now of almost exactly the same weight the point of balance is between the two critical balances. Accordingly the char-

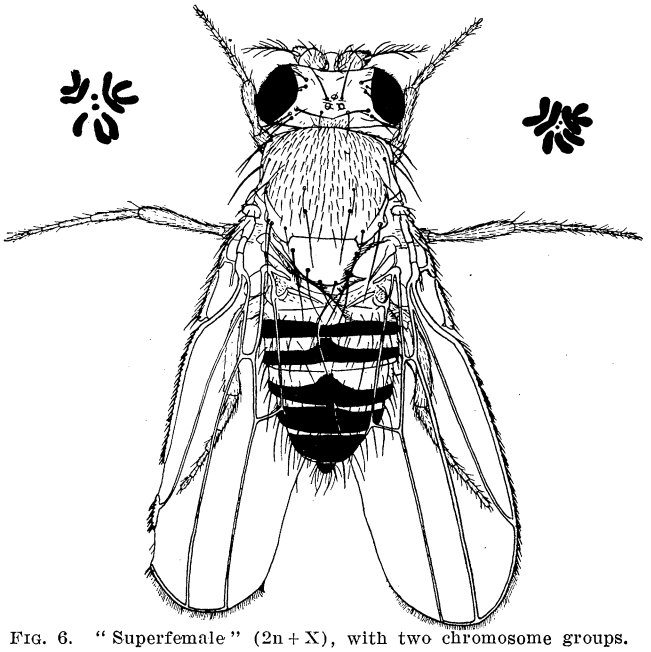

FIG. 6. "Superfemale" (2n + X), with two chromosome groups.

acters of the intersex fluctuate widely with slight environmental differences, and fall into two modes corresponding to the slight difference in balance between two and three of the tiny fourth-chromosomes.

RELATION OF SEX TO CHROMOSOMES IN *Drosophila melanogaster*

Sex		X-chromosomes	Sets Autosomes	Sex Index
Superfemale		3	2	1.5
Female	triploid	3	3	1
	diploid	2	2	1
Intersex	♀-type	2	3(—IV)	.67+
	♂-type	2	3	.67
Male		1	2	.5
Supermale		1	3	.33

The phenomenon of intersexuality might be expected to have a reciprocal phase—namely, supersexes. If the

intersexes result from an intermediate ratio of X to autosomes because the X has a net female tendency, then it might be expected that by increasing the ratio of X to autosomes a superfemale would be produced, and conversely, a supermale by increasing the relative number of

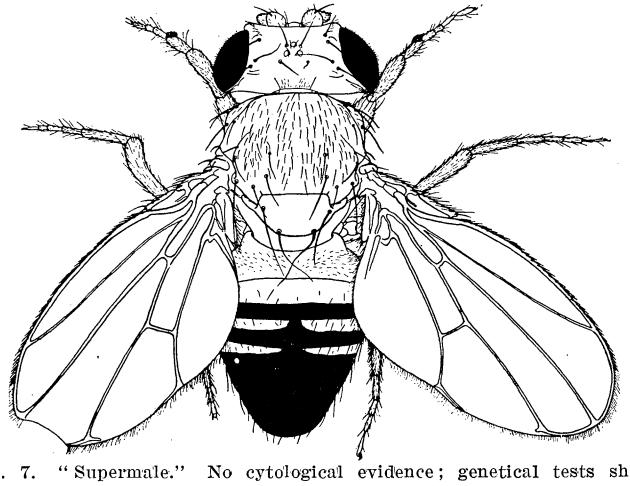

FIG. 7. "Supermale." No cytological evidence; genetical tests show 1X and 3 sets autosomes.

autosomes. Diploid individuals with an extra X-chromosome (2n plus X) have now been identified among the progeny of certain strains of high non-disjunction, among the offspring of triploid females and elsewhere. These flies resemble females but are very inviable and form a distinct character type. They are sterile and sections of the gonads show abnormal ovaries. These differences all result from the unbalance within the X, and are therefore of the sexual-sex-limited category. That these differences are not greater is partly due to the same high internal balance of the X that we met with in analysing males and intersexes, and is partly to be explained on the ground that for many of the characters the overbalance is not yet great enough to pass a second critical point.

Conversely, individuals with one X-chromosome and an extra set of autosomes have been identified among the offspring of triploid females. These are males distinctly different from normal males and sterile.

If there were time, it would be interesting to supplement and modify the view just presented by comparisons with the rich materials elsewhere, and perhaps to speculate as to how this machinery was evolved, and how the genes involved come to expression physiologically.